Dealing with Contamination Issues in NTP Study Materials: Anthraquinone (AQ) as an Example

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Appropriate Scientific Policy

- Accurately and openly present all critical data in study summaries and project titles
- This includes instances where the biological activity of the contaminant may have plausibly affected the outcome of the study

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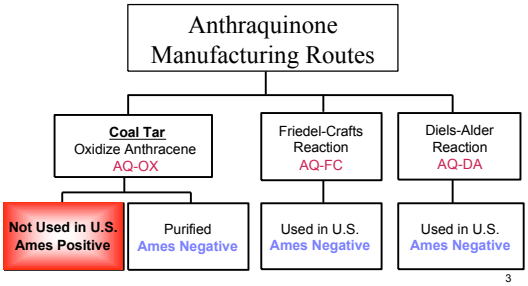
Ames Study Results



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Analysis of AQ Used in the NTP Bioassay

- 0.1% Impurities GC (NTP, 2004 – p.J-2 Abstract)
- 0.5% Impurities HPLC (NTP, 2004 –p.J-2)
  - 9-nitroanthracene (9-NA) at 0.3%
  - a second peak at 0.2% was not identified
- 0.6% Impurities (Int. J. Toxicol. (2004) 23, 335-344)
- Mutagenic activity may reside with several contaminants

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When is it necessary to present contamination issues in the abstract or choice of title?

- When the biological activity of the contaminant may have plausibly affected the outcome of the study

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NTP AQ *Bioassay Material* in TA98 Minus S9

(Butterworth *et al.*, 2001)

Conc. (μg/plate)	AQ (rev/plate)	AQ Purified
0	18	14
30	20	16
60	25	20
125	42*	15
250	62*	10
500	116*	11
1000	213*	15
2000	433*	22

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NTP AQ Study in TA98 Minus S9

(NTP, 2004 – Tables E1 and E2)

Conc. (μg/plate)	AQ (rev/plate)	AQ Purified
0	15	12
33	43*	-
100	70*	11
333	225*	13
1,000	723*	13
2,500	1,497*	12
10,000	-	12

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9-NA Is A Mammalian Cell Mutagen

- Mutagenic in the mouse lymphoma mutagenesis assay (Int. J. Toxicol. (2004) 23, 335)
  - Active with S9
  - As potent as the MMS positive control
  - Primarily small colony mutants suggesting a predominantly clastogenic mechanism
- Mutagenic in a human B-lymphoblastoid cell line that expresses P450 1A1 (Mutat. Res. (1996) 371, 123)

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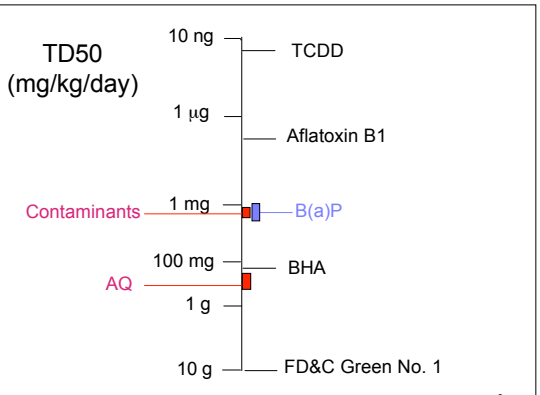

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NTP AQ Test Material

- Kidney/bladder carcinogen in F344 rats
- Liver carcinogen in B6C3F1 mice
- 0.1 – 0.65% impurities including 0.1% 9-nitroanthracene
- Mutagenic in bacterial and mammalian cells
- Contaminants alone plausibly may have produced the tumor response

Purified AQ

- Negative in the following
- Ames mutagenicity
- Mouse lymphoma muta.
- Mutation in Human cell line expressing P450 1A1
- CHO Chromosomal Aberrations
- In vivo mouse micronucleus
- SHE cell transformation assay

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Getting the Science Right

- In NTP TR494 no mention was made of the contamination issue in either the study title or abstract
- The NTP Review Subcommittee appropriately recommended the study title be changed to “Anthracene-Derived AQ” to alert the reader that contamination issues should be considered in evaluating this study (February 17, 2004)

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Regulation of AQ in the US

- Usage of AQ has been and continues to be highly regulated by the EPA and FDA
- Regulatory agencies are competent and experienced in evaluating complex data sets
- Open access to all relevant data only increases the ability to make appropriate decisions

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Appropriate NTP Policy

- NTP's mission statement is to use an "objective, science-based approach in dealing with issues"
- The disclosure of all critical data in an open manner in study summaries and choice of title should be the policy of the NTP
- This particularly applies to situations where the biological activity of the contaminant may have plausibly affected the outcome of the study

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